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NASA TM X-52932



# RECENT ADVANCES IN EMISSION SPECTROSCOPY AND THE DETERMINATION OF TRACE ELEMENTS IN BIOLOGICAL MATERIALS

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TECHNICAL PAPER presented at International Symposium on the Newer Trace Elements in Nutrition sponsored by the U. S. Department of Agriculture
Grand Forks, North Dakota, September 15-17, 1970

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## INTRODUCTION

In discussing the emission spectrometric approach in connection with determination of trace metals in biological materials, one is inevitably drawn to the problem of inadequate detection limits. Certainly, my association with Dr. Hambidge in the determination of trace metals in blood serum, has made it apparent that this is the foremost problem. Therefore, advances in the spectrometric method in the area of detection limits is of primary interest and will be the emphasized in this paper.

The requirement for adequate detection limits, of course, is not the only important requirement for the biological materials. Other aspects of analytical procedures such as accuracy, precision, and economy, for example, can be critically important in the usefulness of a given technique. However, we must first have a signal that we can measure above background noise, and this is too often not the case when measuring trace elements in biological materials.

For purposes of orientation, I will briefly review the methodology of emission of spectroscopy. Figure 1 illustrates the principles of operation. First, the sample must be vaporized under conditions which break chemical bonds resulting in a concentration of free atoms in a gaseous environment. This is most conveniently accomplished in a high temperature environment. In such an environment, the atoms will absorb kinetic energy and "leak" energy in the form of light. In practice, the spectroscopist has used about anything that glows for this purpose including arcs, sparks, hollow-cathodes, plasma-jets, flames, microwave discharges, and lasers.

The light emitted in the excitation source is dispersed in the spectrometer which images atomic "lines" of the elements on the focal curve. (These intensities appear as lines only because that is the geometry of the entrance slit.) Photomultiplier detectors, or alternatively photographic emulsions, are located to intercept the elemental lines. The currents produced by the detectors are proportional to specific elements in the sample. These currents are digitized and ultimately converted to concentrations by means of comparison standards. In principle, any number of parallel detector channels can be included in this arrangement. In the instrument at Lewis there are twenty-two parallel channels which allows the simultaneous determination of as many elements.

The critical steps in the emission procedure depicted in Figure 1 are the sample vaporization and excitation of spectra. The conditions under

which these processes are carried out will almost exclusively determine the precision and detection limits of the analytical procedure. It may be important to have reliable multi-channel spectrometers and automatic data processing for the most effective utilization of the spectral information, but no amount of instrumentation, regardless of cost or sophistication can in itself lower detection limits. The heart of the emission process is in the excitation and it is there where improvements in detection limits can best be approached.

The emission spectrometric procedures that are in use today for the determination of trace metals, vary greatly in experimental detail. Sample sizes vary from a few micrograms to a gram, or so, and the sample may be in liquid or solid form. Sample treatment often entails additions of powdered materials such as lithium carbonate, graphite, and silver chloride, for example. When arcs are used to excite the spectra, currents may vary from a few amperes up to about sixty amperes, and the atmosphere may be air or other gas mixtures under either static or flowing conditions. Almost an infinite variety of combinations of these and other conditions have been used in trace analysis. This work on practical emission procedures has been done by empirically testing various parameters, which have been identified through practice of the art, to give the most favorable results for a specific analysis problem. Conditions established for trace analysis of non-biological materials are not necessarily the best, nor even useful, for biological materials. Therefore, detailed descriptions of specific emission procedures is usually not very instructive, and will be avoided in this paper.

Very little work has been done on the optimization of the total emission process for trace analysis, and for good reason; a theory for the complex emission processes is not available. In recent years, increasing attention has been given to developing a theory for spectrochemical excitation. The first comprehensive book on the subject appeared in 1966 (ref. 1). In reference 2 this theory was applied to quantitative analysis using a direct current arc in air. Since the development of this theory is an important advance in spectrochemical analysis, a brief description of it as it relates to the problem of achieving low detection limits will be presented. By using this theory, even where it is not rigorously applicable, it is possible to discern a continuous thread linking the characteristics of many excitation sources now in use as well as those under development. In this way the rationale for the practical ways to excite atomic spectra, some of which will be discussed, comes into a little better focus. We can then better appreciate the important parameters for obtaining low detection limits and to project the potential of future research.

## THEORETICAL CONSIDERATIONS RELATED TO DETECTION LIMITS

The theory describes the behavior of particles in high temperature plasmas under conditions in which the electrons, ions, and atoms are at thermodynamic equilibrium in the volume under observation. This condition prevails when random collisions of particles caused by thermal agitation is the only important means for transferring energy among the particles. Although it is rare when this model is rigorously applicable, it is roughly applicable to metal atoms in arcs, microwave discharges, lasers, and flames.

The relations for the atomic emission intensity from a neutral atom in a high temperature environment is shown in equation (1).

$$I \cong (1- \alpha)\, N_o A g \nu EXP - \Delta E/kT \qquad (1)$$

where:

| | |
|---|---|
| $I$ | emission intensity |
| $N_o$ | atom number density |
| $Ag\nu$ | atomic constants descriptive of given element and transition |
| $\Delta E$ | energy of transition |
| $k$ | Boltzman constant |
| $T$ | plasma temperature |
| $\alpha$ | fraction of atoms ionized |

From this relation we see that maximum line intensities that are necessary for low detection limits, are achieved when; (1) the maximum number of neutral atoms is supplied to the excitation zone, and (2) when the plasma temperature is optimum. Surprisingly, the first condition is the most difficult to achieve experimentally. Unfortunately, the simple expedient of vaporizing more sample, is not usually the answer. Introducing large densities of atoms into a high temperature environment is a fundamental problem in achieving low detection limits.

I mentioned that the temperature must be an optimum one. The emission intensity increases with temperature, but if the temperature is too high the neutral atoms will be depleted by ionization resulting in loss of emission from the neutral states. Figure 2 shows a graphical form of this equation for various ionization potentials and excitation potentials as a function of plasma temperature. It can be seen from the figure that for a given spectral line, characterized by the ionization potential of the element and the excitation potential for the spectral line, there is an optimum temperature for maximum line emission.

As an aid in orientation with respect to the temperature scale of figure 2, it should be noted that flame temperatures lie below 5000° K, whereas arcs, lasers, and microwave discharges produce temperatures from 5000° K to over 10,000° K. As is indicated on the figure, the newer trace elements

such as Ni, V, Cr, and Sn are optimally excited in the temperature range between 5000° K and 7000° K. The optimum temperature for Se, however, is much higher than temperatures reached in most discharges in common use.

There is an aspect of this model which must now be considered because it can be of vital importance with biological materials. The temperature of the plasma is determined by the atomic composition of that plasma. So it isn't simply a matter of introducing large numbers os sample atoms into a plasma t fixed temperature. By doing so, we may drastically alter the temperature. However, it is possible to adjust the composition of the sample so that an optimum temperature results. The modifying effect of sample atoms on the excitation temperature explains much of the problem of matrix-effect, and also explains why spectrographers are fond of mixing compounds known as buffers with their samples. Bearing in mind that many biological materials are rich in alkali metals, and that easily ionized elements effect a greater change on excitation temperatures, we can see that this is an important consideration in obtaining low detection limits for transition elements in biological materials. Unless the sample composition is ideal, the resulting plasma temperature will not be the optimum one.

Having briefly considered the effect of line intensity on detection limits, let us now consider another equally important aspect. Namely, what are the important parameters for obtaining minimum background intensities. The detection power of the excitation source can be expressed in terms of a ratio between the line intensity to the background intensity at the same spectral wavelength. (More precisely, the ratio should be with respect to the variability of the line and background. However, for practical purposes we assum that for a given excitation source, the lower the background the lower the variability in background in direct proportion.)

The background from most high temperature plasmas is due to two basic causes; (1) radiation emitted by an electron losing energy as it is deflected by a positive ion (bremstrahlung), and (2) energy emitted as a result of recombination of an ion and an electron. In this discussion we do not consider light emitted from incandescent electrodes, nor do we consider band spectra. These sources of background are not inherent in the plasma and can be controlled experimentally.

One expression for the continuum emitted as a result of electron-atom interactions in an arc is given in reference 1, and shown in equation (2).

$$I_b = n_e n_i (kT)^{1/2} \text{ EXP } - (\nu_l - \nu)/kT \qquad (2)$$

where:

| | |
|---|---|
| $I_b$ | intensity of background |
| $n_e$ | electron number density |
| $n_i$ | ion number density |
| k | Boltzman constant |
| T | plasma temperature |
| $(\nu_l - \nu)$ | frequency dependent parameter |

Although this relation agrees with experiment only qualitatively, it is of interest to consider the parameters that might be manipulated to obtain minimum background. From equation (2) we conclude that as long as electrons and ions are present in the plasma we will have to contend with background. However, we do notice a difference in the dependency of line and background intensities on particle density. Whereas the line intensity was directly proportional to particle density (eqn. (1)), the background is proportional to the square of particle density (eqn. (2)). The latter follows because at electrical neutrality $n_e = n_i$, or $n_e n_i = n_e^2 = n_i^2$. Therefore, as we reduce particle density, the background goes down faster than the line intensity. This suggests that thermal sources operated at lower particle densities are more advantageous for obtaining low detection limits than sources operated at higher particle densities. Although it is feasible to manipulate particle densities independently of plasma temperature, this is not easy to do without adversely affecting the maximum rate of sample introduction, thus resulting in loss of line intensity. The search for the optimum excitation conditions yielding the lowest detection limits is where theory ends and experimental work begins.

In summary, the three most important aspects of any emission spectrometric procedure designed to detect the smallest amounts of elements are; (1) high rates of sample introduction into the excitation zone, (2) optimum excitation temperature, and (3) minimum background emission. The emission techniques with the lowest detection limits are compromises of the best combination of these three properties. A systematic development of excitation techniques with vastly improved detection limits must await further developments in excitation theory. In the meantime, slow but positive progress is being made by empirical experimental evaluations. Now let us consider some new excitation sources which are illustrative of this progress.

## RECENT INNOVATIONS IN PRACTICAL EXCITATION TECHNIQUES

Three practical approaches for exciting atomic spectra will be described. The first procedure to be described was developed at the Lewis Research Center and involves the excitation of samples in an argon atmosphere in the presence of relatively large amounts of silver chloride vapor. This technique is hereafter referred to as the Argon-Silver-Arc. This procedure

has been used routinely at the Lewis Laboratory (ref. 3), and is incorporated into an automated spectrometric facility. It has also been used by Hambidge, reference 4, for the determination of chromium in ashed blood serum and hair.

Two newer techniques that have recently been reported will also be described. These are both based on microwave excitation under conditions that have resulted in uncommonly low detection limits.

## Argon-Silver-Arc Technique

In this procedure, samples are put into solution and a ten-microliter aliquot of the solution is deposited on a carbon rod and dried to form a residue. In addition to containing the sample residue, the carbon rods also contain a few milligrams of silver chloride. The carbon rod serves as the anode in a direct-current arc operated in a static argon atmosphere. The sample residue is evaporated along with the silver chloride under conditions which allow detection of nanogram amounts of many metal elements. The method is therefore applicable to micro-analysis (very small samples) as well as to trace analysis (low concentrations). The advantages of this arcing procedure result from the enhancement by silver chloride on line entensities, elimination of band spectra, induced stability of the arc discharge by a special cathode design, and a special anode design.

This arcing procedure has been integrated with an automated spectrometer to provide rapid and automatic analyses of ten samples in the arc chamber. Figure 3 summarizes the procedural steps of the automated procedure. As applied to biological materials, the material is ashed in a low temperature asher and dissolved in dilute hydrochloric acid. The concentration of ashed blood serum is 1.3 milligrams per 10 microliters in 6N HCl. A ten-microliter aliquot of this solution is added to the carbon electrode containing 4mg of AgCl, and dried for a few seconds at 90° C. The silver chloride is added to batches of carbon electrodes by first doping with 10 microliters of silver nitrate solution followed by addition of 10 microliters of hydrochloric acid solution. The solution concentrations are such that 4 milligrams of silver chloride are precipitated in the carbon matrix. After drying the sample solution, an intimate mixture is formed between the sample residue and the silver chloride. In addition to modifying the excitation conditions in the argon arc, the silver chloride also serves to provide a high halide activity during sample vaporization to prevent carbide formation which can otherwise cause serious analytical errors. The pointed anode was designed to introduce relatively high rates of solids without disturbing the arc stability. The excitation temperature of this arc has not been measured but temperatures of similar arcs are about 6000° K.

A batch of eleven electrodes prepared in this way are loaded into a gas tight arc chamber and arced in a completely automated sequence. The intensities from as many as twenty-two elements are simultaneously recorded on punched paper tape and converted to absolute micrograms of elements by means of calibration curves stored in the computer memory. With this procedure about 16 elements including, sodium, potassium, iron, phosphorous, silicon, strontium, calcium, magnesium, copper, zinc, chromium, molybdenum, nickel, manganese, aluminum, and vanadium can be detected in 1.3 milligrams of ashed blood serum. Cobalt is not detected. However, the elements molybdenum, nickel, and vanadium are marginally detected and probably could not be measured precisely without further improvements in detection limits. Since these arcing conditions were established for metallurgical work, additional development work aimed at optimizing conditions for biological materials is indicated.

## Low Power Inductively-Coupled Microwave Discharge Technique

This discharge has been of interest to spectroscopists as an excitation source for a number of years. Recently, the discharge was operated using conditions that resulted in some very good detection limits (ref. 5). The excitation assembly, without the power supply, is shown in Fig. 4. In operation, the glow capillary is inserted into the excitation cavity. The samples are introduced by drying a few microliters of solution on a platinum wire filament. The sample is then vaporized by applying current to the filament. A flow of argon sweeps the sample vapor into the excitation zone where compounds are dissociated and the atomic spectra is excited. The power supply for this technique is similar to the familiar diathermy unit which operates at 2450 MHz. The excitation temperature of the plasma formed inside the capillary is in excess of 5000° K and has been reported at high as 10,000° K. Limits of detection as low as $10^{-11}$ to $10^{-12}$ grams were reported with this source in reference 5. The superior detection limits presumably result from the efficient introduction of sample atoms into the high temperature plasma. Some aspects of this method for trace and micro analysis will be summarized at the end of the talk.

## High Power Inductively-Coupled Microwave Discharge (Plasma Torch) Technique

This excitation source can be thought of as a scaled-up version of the source just described. It operates at a power level of a few thousand watts, or about 50 times the power of the diathermy source, and at a frequency of 30 MHz. It also operates in flowing argon and produces excitation temperatures in the region of 10,000° K. This source has been under evaluation as an emission source for the past few years. Recently, a more efficient way was discovered of introducing liquid samples which resulted in vastly improved detection limits (ref. 6). Figure 5 shows how increased

sample introduction was achieved. The torch is operated under conditions which open a hole in the center of the plasma and this allows the liquid sample to be introduced at a higher rate than was previously possible. In addition, the authors used a rather elaborate arrangement to sonically nebulize and desolvate the liquid droplets prior to entry into the plasma. Apparently the single most important factor which previously limited the detectability of this source was the rate of sample introduction. The further development of this excitation source will be of interest to those interested in trace metals in biological materials.

## CONCLUDING REMARKS

Table I provides a summary comparison of detection limits for the techniques we have discussed and also some detection limits by flame spectroscopy and laser excitation. I have made estimates from the literature which I consider to be typical for the newer trace elements such as Cr, Ni, V, and Mo. These estimates are only within about a factor of ten because of variable definitions of detection limits. For the flame and high powered microwave discharge, detection limits are determined using the best experimental conditions for each element and are, therefore, not necessarily applicable to simultaneous element determinations. For the other sources the experimental conditions were the same for all elements.

The absolute detection limits given in table I are a measure of the detection power for micro-analysis. These absolute detection limits are not necessarily directly convertible to trace detection limits because of the effect of other elements also present in the samples. Therefore, to estimate the detection power for trace analysis in liquid or solid samples, we must know the effect of matrix elements on the spectral intensities. For those techniques where this information is known, we can give an estimate of trace detection limits, otherwise no estimate is given. The use of a dashed line in table I indicates that a numerical estimate would not be meaningful but does not necessarily mean that the technique is inapplicable to the particular sample form, i.e., solid or liquid. Also listed in table I are the sample amounts required for analysis and the reagent blank. The reagent blanks are classified on the basis of volume of reagent used. Although reagent blanks can be corrected to some extent, they are limiting at progressively lower concentration.

The detection limit data in table I illustrates the progress that has been made in emission spectroscopy in detecting smaller quantities of metals and metals at lower concentrations. In evaluating these and other techniques for the determination of trace metals in biological materials, other characteristics of the techniques listed in table I should also be taken into consideration.

Further progress in lowering detection limits will continue to be concerned with optimization of excitation conditions. The rate of progress in this work is hindered by lack of a quantitative theory of the emission processes. Although quantitative description of the emission processes is the foremost unsolved problem in emission spectroscopy, progress in this direction will greatly benefit the development of practical analytical procedures with lower limits of detection.

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Table I

COMPARISON OF TYPICAL POWERS OF DETECTION OF SOME EMISSION SPECTROMETRIC SOURCES[a]

| | LOW POWER MICROWAVE PLASMA (b) | HIGH POWER MICROWAVE PLASMA (c) | LASER (d) | ARGON-Ag-ARC (e) | FLAME |
|---|---|---|---|---|---|
| ABSOLUTE LIMIT, g | $10^{-11}$ | $10^{-9}$ | $10^{-9}$ | $10^{-9}$ | $f_{10^{-8}}$ |
| LIMIT IN LIQUIDS, ng/ml | ---------- | 3 | ---------- | ---------- | 5-1000 |
| LIMIT IN SOLIDS, ppm | 1-10 | ------ | 1-10 | 0.2 | ------ |
| REAGENT BLANK | LOW | HIGH | LOW | LOW | HIGH |
| QUANTITY OF SOLID SAMPLE AT DETECTION LIMIT | µg | ------ | µg | µg | µg |
| LIQUID | MICROLITERS | LITERS | MICROLITERS | MICROLITERS | ml |

[a]AUTHOR ESTIMATES.
[b]RUNNELS & GIBSON.
[c]DICKENSON & FASSEL.
[d]BRECH.
[e]GORDON.
[f]$10^{-10}$ IN GRAPHITE FURNACE.

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EMISSION SPECTROSCOPY
PRINCIPLE OF OPERATION

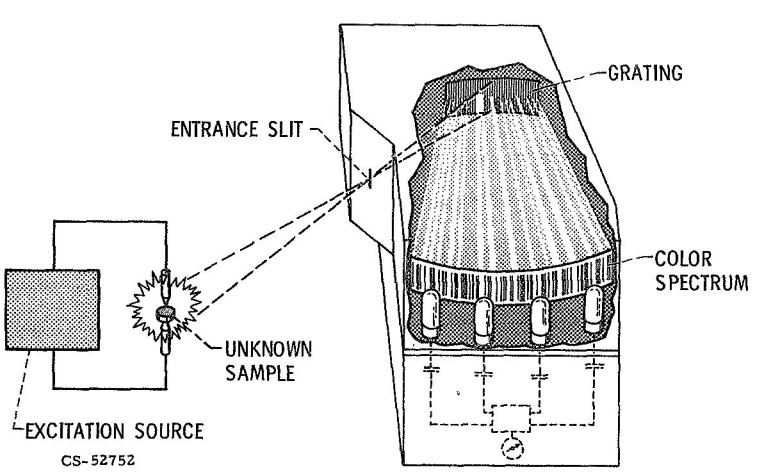



Figure 1

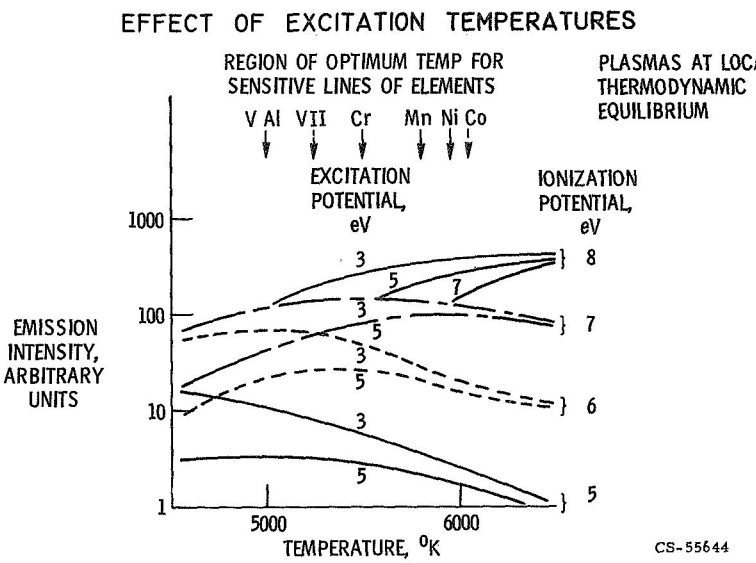



Figure 2

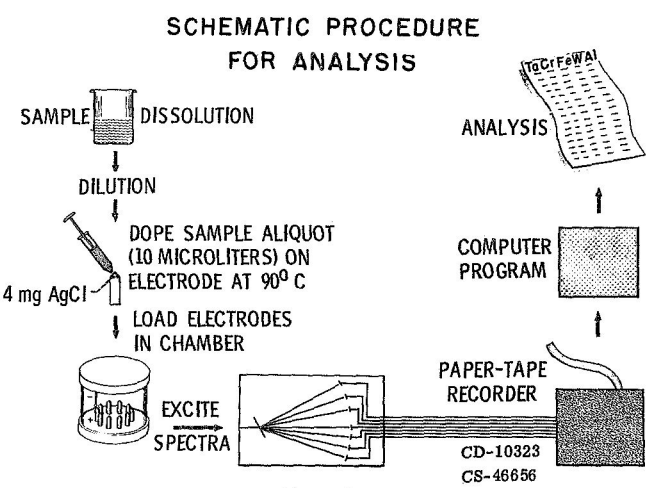



Figure 3

MICROWAVE EXCITATION ASSEMBLY

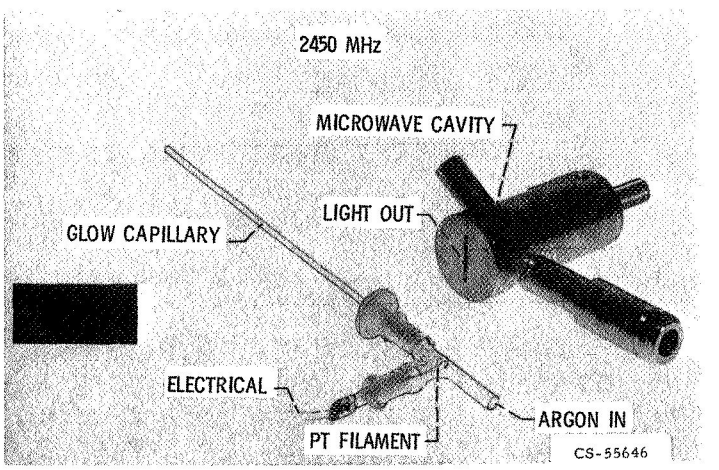
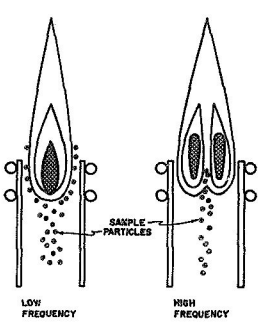



Figure 4

AEROSOL ENTRY INTO UNIFORM VS TOROIDAL SHAPED PLASMA




DICKENSON & FASSEL (ref. 6).

Figure 5